

Studying Laser tissue interaction of two internal organs of rat

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Abstract— In the recent paper, the laser tissue interaction of two different organs tissues of mouse can be studied when these tissues are exposed to different types of laser. In this paper we show how the 405nm, 532nm, 785nm and 1064 nm lasers are intimately affecting on the surface and the histology of these organs. The effects are related to wavelength, the power and energy of the exposed laser.

Firstly, we will outline important laser–tissue interaction during laser irradiation. The process of photon absorption and thermal energy diffusion in the target tissue and its surrounding tissue are crucial. Such information allows the selection of proper operating parameters such as laser power and exposure time for optimal tissue effect.

Different tissue configurations are used in the study.

Index Terms— mouse's liver, mouse's spleen, histology, absorption spectrum of the organs, many laser types.

I. INTRODUCTION

In order to understand how to select the ideal of laser from the myriad of currently available devices of treatment of any cutaneous condition it is important to first understand how light produces a biologic effect in tissue. The interaction of laser light with living tissue is generally a function of wavelength of the laser system. [1].

Laser light entering the biological tissue is either scattered or absorbed. Scattering is a process by which energy in a beam is redirected without a change in its wavelength. The new direction of the emitted beams from the surface of the refracting particles depends on the size and the shape of the molecules in question as well as the wavelength of the radiation. In general scattering and absorption affect the distribution of photons in the tissue target, but absorption alone, determines the effect of radiation [2]. If the light is reflected from the surface (scattering) of the tissue or transmitted completely through it without any absorption, then there will be no biologic effect. In other words, in order for laser energy to produce any effect in tissue, it must be first absorbed. Absorption is a transformation of radiant energy (light) to a different form of energy usually heat by specific interaction with tissue [1]. Absorption of photon may alter the electronic structure of molecules [2].

II. LASER TISSUE INTERACTION MECHANISMS:

Laser effect in biological tissues may be divided in five categories: (1) photochemical, (2) thermal, (3) photoablation

(4) plasma-induced photoablation (5) photodisruption [2, 3]. Figure (1) shows how the five interaction mechanisms depend on the duration of the light exposure and the irradiance (fluence rate), i.e. the light energy delivered per unit area per unit time, the power per unit area, in W/cm^2 . Which in Figure (1), a double-logarithmic graph with the five basic interaction types is shown. The ordinate expresses the applied power density in W/cm^2 . The abscissa represents the exposure time in seconds. Two diagonals show constant energy fluences at 1 J cm^{-2} and 1000 J cm^{-2} , respectively. Notice that both axes are log-axes, i.e. the log of the irradiance increases linearly on the vertical axis, and the log of the time increases linearly on the horizontal axis. [3, 4].

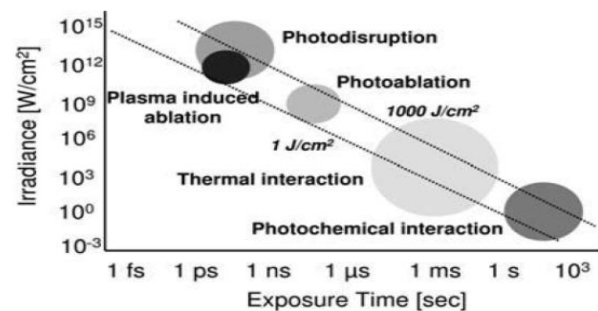


Figure (1): Laser–tissue interaction [4].

This plot shows fluence rate versus interaction time (or pulse length) for a variety of medical applications:

1. Photochemical reactions: when a molecule absorbs a photon of sufficient energy, the energy can be transferred to one of the molecule's electrons. An electron with higher energy can more easily escape the nuclear forces keeping it close to the nucleus, and so excited molecules (which are molecules with an electron in a higher energy state) are more likely to undergo chemical reactions (exchanging or sharing of electrons) with other molecules. In photodynamic therapy, for instance, a photosensitising drug (a concoction of molecules which, when they absorb light, cause reactive oxygen species to form) is used to cause necrosis (cell death) and apoptosis ('programmed' cell death).

Photodynamic therapy is increasingly widely used in oncology to destroy cancerous tumours.

2. In photothermal interactions, the energy of the photons absorbed by chromophores (a term used to refer to any light-absorbing molecules) is converted into heat energy via molecular vibrations and collisions, which can cause a range of thermal effects from tissue coagulation to vaporization. Applications include tissue cutting and welding in laser surgery, and photoacoustic imaging.

3. In photoablation, high-energy, ultraviolet (UV) photons are absorbed by electrons, raising them from a lower energy 'bonding' orbital to a higher energy 'non-bonding' orbital, thereby causing virtually immediate dissociation of the

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molecules. This naturally leads to a rapid expansion of the irradiated volume and ejection of the tissue from the surface. This is used in eye (corneal) surgery, among other applications.

4. In plasma-induced photoablation a free (sometimes called 'lucky') electron is accelerated by the intense electric field which is found in the vicinity of a tightly focused laser beam. When this very energetic electron collides with a molecule, it gives up some of its energy to the molecule. When sufficient energy is transferred to free a bound electron, a chain reaction of similar collisions is initiated, resulting in plasma: a soup of ions and free electrons. One application of this is in lens capsulotomy to treat secondary cataracts.

5. photodisruption, are the mechanical effects that can accompany plasma generation, such as bubble formation, cavitation, jetting and shockwaves. These can be used in lithotripsy (breaking up kidney or gall stones), for example [3].

Absorption depends on the concentration and absorption spectra of specific molecules in the tissue [5]. Photons from infrared radiation differ from ultraviolet and visible light radiation because they deposit their energy by exciting the molecular electrons to higher energy levels, and also they can directly transfer energy to the vibrational energy levels of the irradiated molecules. In general, molecules in biological tissues are opaque to ultraviolet radiation at wavelengths shorter than 300 nm and in this range of wavelength usually cause photochemical reactions in biological tissue [1, 6]. While in the range between (300-400) nm only a limited number of bio-molecules have moderate absorption. Most bio-molecules are effectively transparent between (400-1300) nm. But these molecules have a strong vibrational absorption bands for infrared radiation at wavelength greater than 1300 nm. Difference in spectral absorption properties of different molecules permits selective damage to specific components of a target tissue [3]. In general, proteins molecules have a high absorption of ultraviolet radiation, but hemoglobin, melanin, and other pigments have specific features of absorption the visible radiation, while infrared radiation can be absorbed by the water molecules in the bio-tissues. The spectrums between (700-900) nm have maximum penetration in the tissue [6].

Thermal effects are perhaps the most widely encountered form of laser tissue interaction in clinical practice [4]. Laser radiation at wavelengths produces usually a thermal effect. Thermal effects occur when photon absorption by the outer electrons or molecular vibrations produces enough temperature rises to denature the biomolecules and the weak van der Waals forces that help to stabilize their structures. Thermal tissue damage requires 10° to 20° increase in retinal temperature, which the extent of thermal injury is proportional to the magnitude and duration of a temperature increase also temperature rises in irradiated tissue is proportional to light absorption in tissue, which in turn is determined by how effectively its constituent molecules absorb incident photon of a particular wavelength [1].

III. MATERIALS METHODS:

In order for light to affect tissue, absorption must take place. The initial deposition of energy depends on the tissue optical properties and the irradiation conditions. Also, the

evolution of temperature with time depends on the thermal properties of the tissue [5].

Alternatively, When a molecule is exposed to high intensity radiation, then it can be raised to an excited state from which a variety of chemical reactions are possible such as the generation of free radicals and reactive oxygen species [5]. Then its ground state will decay due to multiphoton ionization. When the photoemitted electrons are energy analyzed they produce a spectrum containing a series of peaks, separated by the photon energy [4].

The mechanical properties of the tissue govern the propagation of exposed light and their biological effect. This emphasizes the point that is the rate of energy absorption that determines the nature of the light-tissue interaction [5].

The dominant mechanism will depend on:

1. The type of molecules the tissue is made of and contains. (These determine the energy levels - the energies of photons that can be absorbed and the available de-excitation pathways, i.e. the routes through which the energy leaves the state into which it was absorbed, to end up as heat or perhaps another photon.)
2. The frequency (or wavelength) of the light, i.e. the energy associated with each individual photon.
3. The power per unit area delivered by the laser.
4. The duration of the illumination, and repetition rate of the pulses for a pulsed laser [3].

By carefully choosing the laser characteristics the interaction can be restricted to a specific mechanism, and therefore a specific effect on the tissue. Lasers are therefore useful for medical applications [3].

Four different laser wavelengths with different powers can be used in the present work. In the following, a summary is shown for the different types of laser that can be used in our work.

A. 405 nm LASER DIODE:

The violet laser diode at wavelength of (405 nm) can be used in Medical diagnostics [7]. This laser was used in the present study with power of 98 mW, with spot size of 4 mm which this laser was shined on the tissue for 5 sec at a distance of 25 cm.

B. SHG Nd:YAG LASER:

The KTP laser produces green light at 532 nm, which is well absorbed by hemoglobin melanin but penetrates relatively superficially. It has a higher incidence of mild side effects due to epidermal injury. This wavelength of laser is limited to treating epidermal pigmented lesions [1]. Our laser is Q-switching laser with energy equal to 1000 mJ with repetition frequency equal to 1 kHz and spot size equal to 4 mm, this laser was exposed to the living tissue with 5 sec at a distance of 1 cm.

C. 785 nm LASER DIODE:

This wavelength of Q-switching laser is effective at removing black, blue, and most green tattoo inks, and less proficient at removing red or orange inks [1].

In general, these CW lasers, when used by skilled operators, are effective in the treatment of epidermal pigmented lesions. Also this laser is used in Medical laser therapy [1,8].

In our work, the CW 785 nm laser diode, with power of 2.5 mW and spot size equal to 4 mm is shine to mouse organ tissues for time equal to 5 sec at a distance of 1 cm.

D. CO₂ LASER:

The carbon dioxide laser emits infrared light at 10.600 nm, which is absorbed by tissue water. The major application of CO₂ laser is used in surgery. This laser destroys the superficial skin layers nonselectively and can be used to remove superficial epidermal pigment, especially seborrheic keratosis [1,8]. This laser is shine to the tissue of power equal to 10 W with spot size equal to 4 mm. This laser is exposed to the tissue for 5 sec at a distance equal to 25 cm.

IV. RESULTS:

Here we show that when two different organs of mouse are exposed to many different lasers wavelength, what these causes in these tissues are.

In general, all used laser were affected on the surface of the two tissues but in different level, which these lasers were leaved a mark on the surface of these tissues, this mark is like a burn of the skin.

The 405 nm, 532 nm, and 785 nm lasers were leaved a white mark on the tissue but this mark was different in the level and size from one tissue to other and from one laser to other. The effect of the 532 nm laser was the most popular effect from the other two lasers.

But, CO₂ laser was the higher affect from other the lasers, which this laser was leaved a black mark on the surface of tissue.

The other results are the histological study of the tissue and the effect of these lasers on the histological of the liver and spleen tissues of rat.

A. LIVER

The normal structure of mouse's liver shown in the figure (2). This section showing the normal structure appearance consist a lobule central vein and sheets of hepatocyte cell.

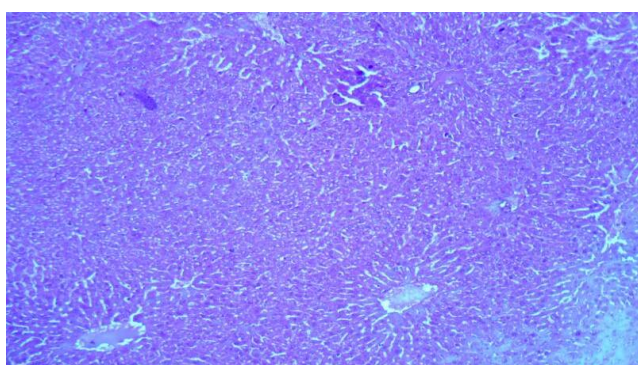


Figure (2): the normal structure of rat's liver tissue.

The absorption spectrum diagram of liver can be measured using spectrophotometer (to measure the spectrum range from (200-1100) nm) and FTIR (to measure the spectrum in IR). From figure (3), quite clearly that the liver have many absorption peaks, and it have low absorbance in UV range, but the higher absorbance of liver can be in 648 nm. While figure (4) show the high absorbance of the IR.

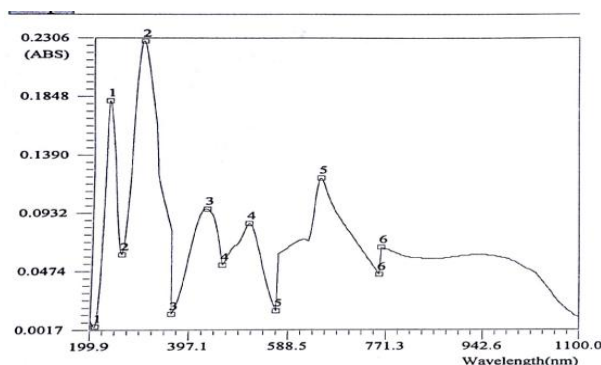


Figure (3): absorption spectrum diagram of the liver tissue as measured in spectrophotometer.

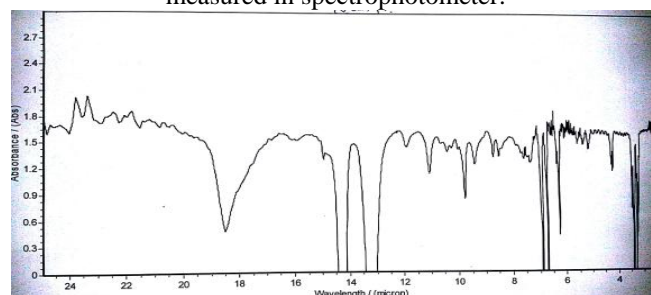


Figure (4): FTIR spectrum of live tissue.

The liver tissue can be exposed to different lasers:

a) 405 nm LASER DIODE:

When liver's tissue exposed to this laser at a distance of (25 cm) and in time of (5 sec), then structure of liver tissue shown in figure (5) and figure (6). In these figures, the section showing the look-like normal structure look-like with sinusoidal dilatation.

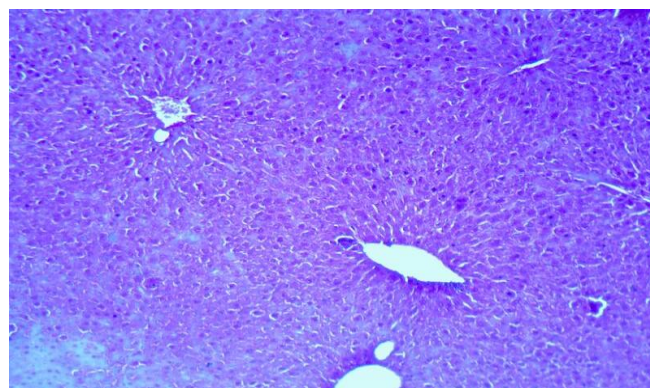


Figure (5): section of liver tissue can be exposed to 405 laser diode (magnification x 200).

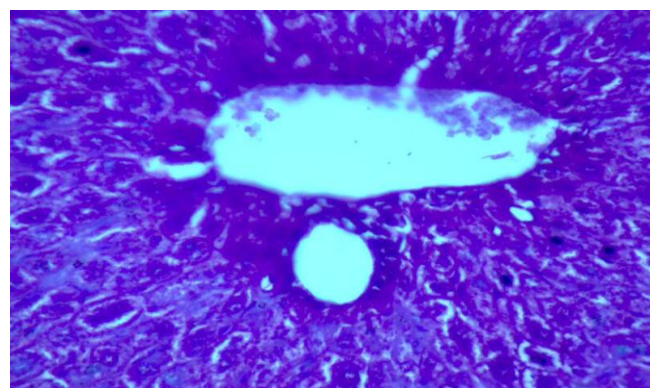


Figure (6): section of liver tissue can be exposed to 405 laser diode (magnification x 400).

b) SHG Nd:YAG LASER:

When the liver tissue was exposed to SHG Nd:YAG laser at a distance of (1 cm) and in time of (5 sec), then the structure of the liver tissue shown in figure (7) and figure (8) and figure (9).

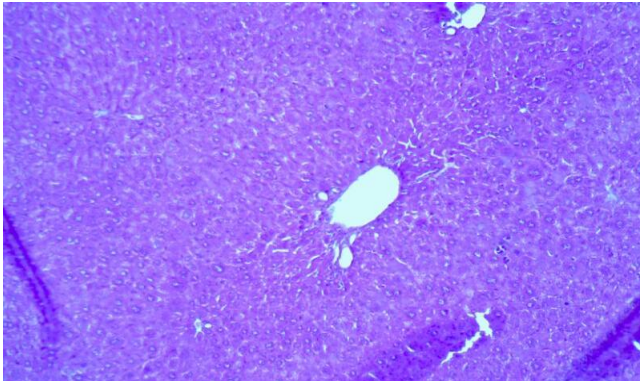


Figure (7): section of liver tissue can be exposed to 532 nm laser (magnification x 100).

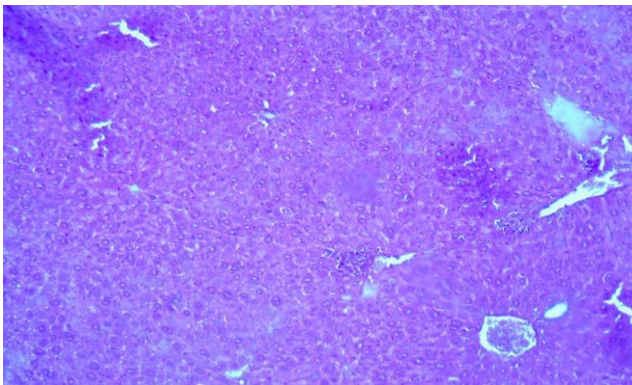


Figure (7): section of liver tissue can be exposed to 532 nm laser (magnification x 200).

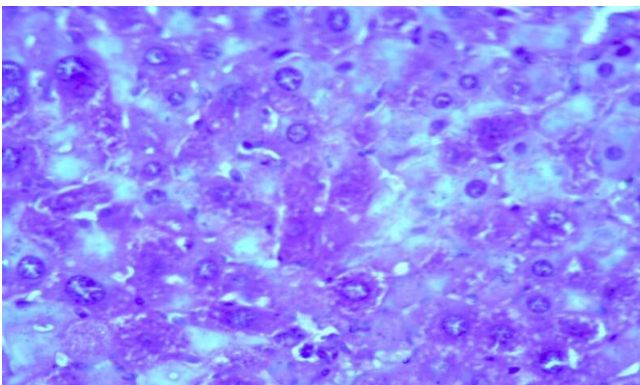


Figure (9): section of liver tissue can be exposed to 532 nm laser (magnification x 400).

Quite clearly, these sections in the pervious figures are showing the local discrete necrosis of parenchymal tissue with inflammatory cells infiltral.

Also, the section of liver in figure (7) showing certain degenerative and discrete necrotic cell.

c) 785 nm LASER DIODE:

When liver's tissue is exposed to N-IR laser diode of 785 nm wavelength at a distance of 1cm, and with time of (5 sec), then the tissue of the liver is showing in figure (10) and figure (11) and figure (12).

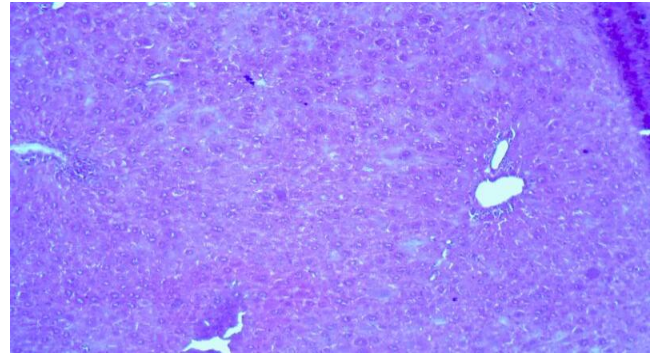


Figure (10): section of liver tissue can be exposed to 785 nm laser (magnification x 100).

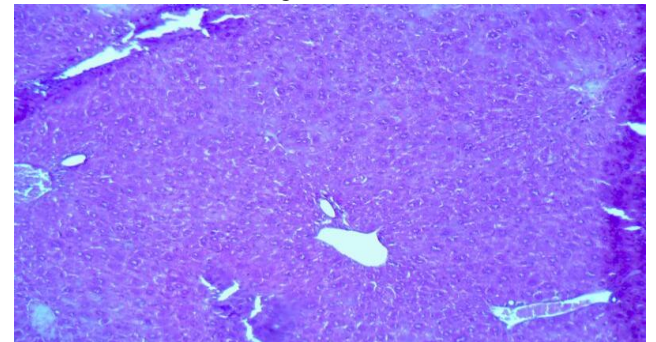


Figure (11): section of liver tissue can be exposed to 785 nm laser (magnification x 200).

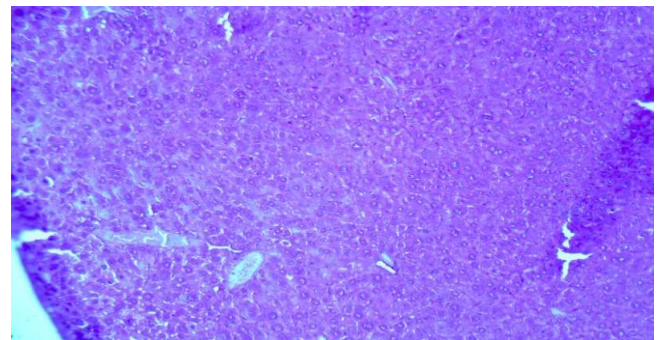


Figure (12): section of liver tissue can be exposed to 785 nm laser (magnification x 400).

From there figures, these sections are showing the look like normal structure with local necrosis and inflammatory.

d) CO₂ LASER:

The liver's tissue is shine with a CO₂ laser at a distance of (25 cm) and with time of (5 sec), which the exposed tissue is shown in figure (13) and figure (14) and figure (15).

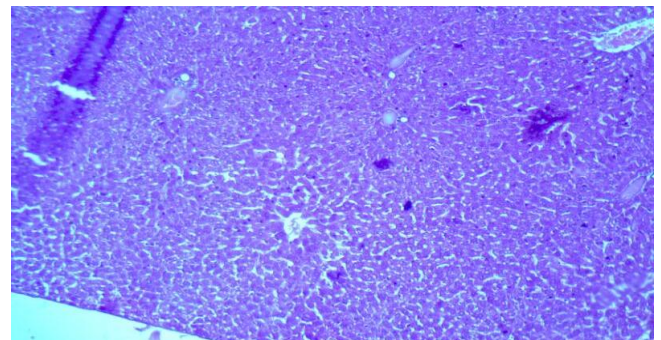


Figure (13): section of liver tissue can be exposed to CO₂ laser (magnification x 100).

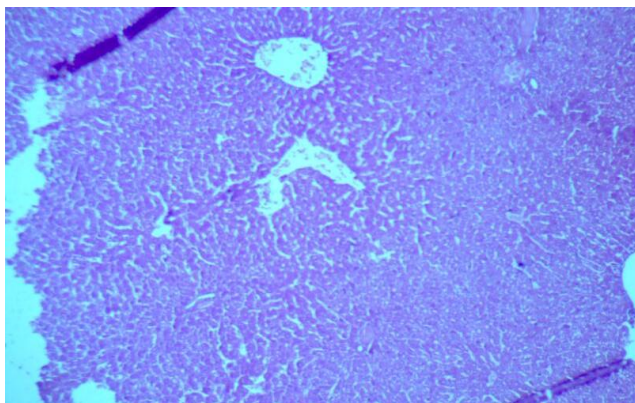


Figure (14): section of liver tissue can be exposed to CO₂ laser (magnification x 200).

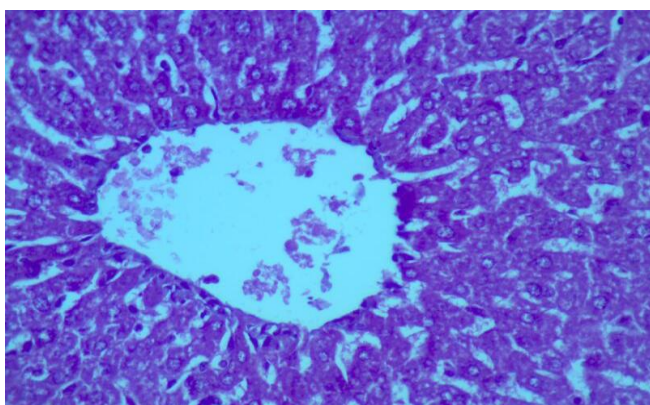


Figure (15): section of liver tissue can be exposed to CO₂ laser (magnification x 400).

From these figures, these sections are showing the look like normal structure with sinusoidal dilatation.

In figure (15) with the large magnification, it is clearly show central vein with sheet of hepatocytes and sinusoidal dilatation.

B. SPLEEN:

The normal structure of rat's spleen is shown in the figure (16).

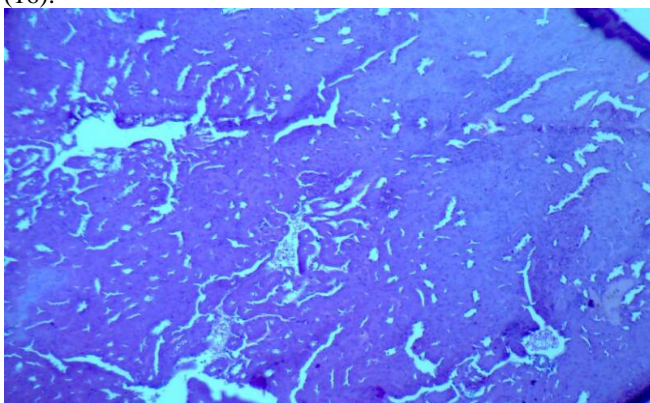


Figure (16): the normal structure of rat's spleen tissue.

The absorption spectrum diagram of spleen can be measured using spectrophotometer (to measure the spectrum range from (200-1100) nm) and FTIR (to measure the spectrum in IR). From figure (17), quite clearly that the spleen have many absorption peaks, and it have high absorbance in UV range, which the higher absorbance of spleen can be in 302 nm. While figure (18) show the high absorbance of the IR.

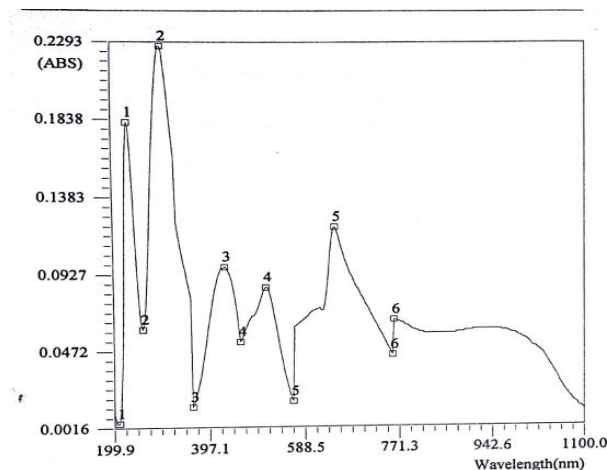


Figure (17): absorption spectrum diagram of the liver tissue as measured in spectrophotometer.

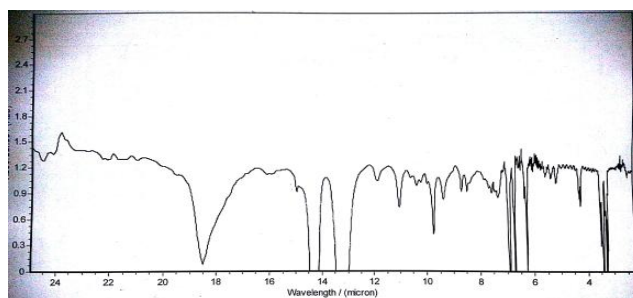


Figure (18): FTIR spectrum of live tissue.

a) 405 nm LASER DIODE:

When spleen's tissue exposed to this laser at a distance of (25 cm) and in time of (5 sec), then structure of spleen tissue shown in figure (19) and figure (20) and figure (21). Which in these figure, it quite clearly, that the sections are showing degenerate and necrosis of parenchymal splenic tissue.

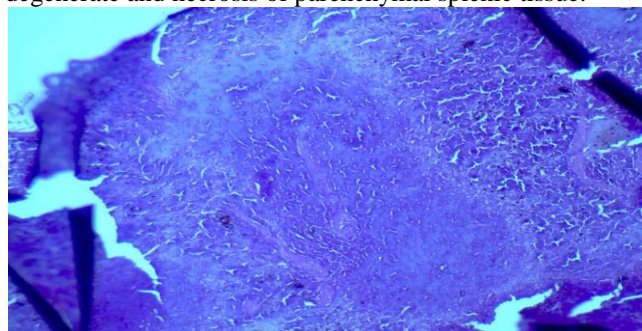


Figure (19): section of spleen tissue can be exposed to 405 laser diode (magnification x 100).

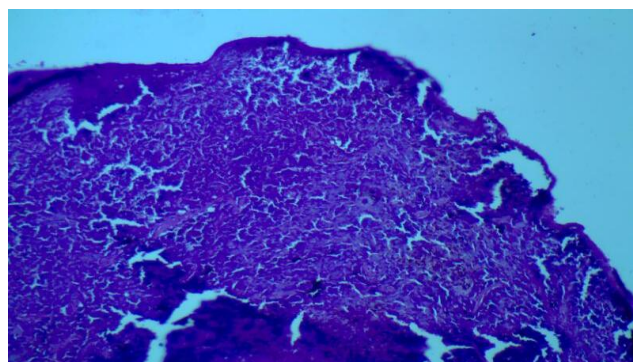


Figure (20): section of spleen tissue can be exposed to 405 laser diode (magnification x 200).

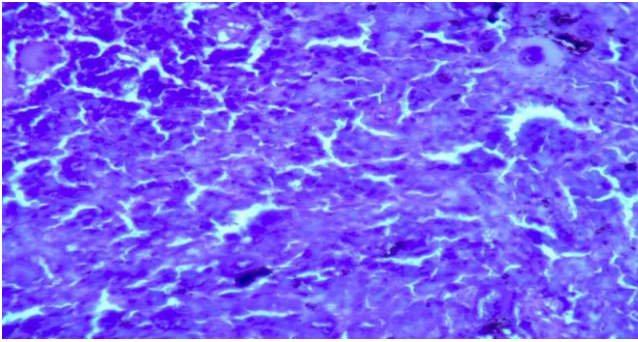


Figure (21): section of spleen tissue can be exposed to 405 laser diode (magnification x 200).

b) SHG Nd:YAG LASER:

When the spleen tissue was shine by SHG Nd:YAG laser at a distance of (1 cm) and in time of (5 sec), then the structure of the spleen tissue shown in figure (22) and figure (23) and figure (24).

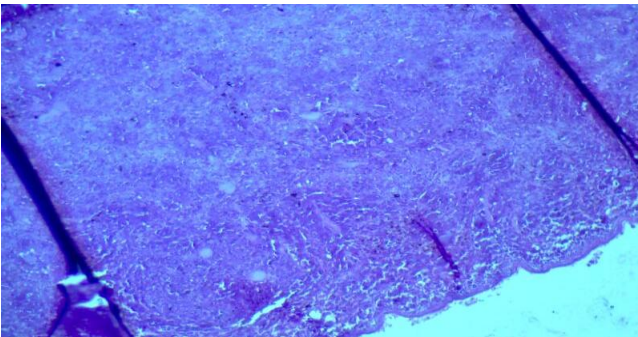


Figure (22): section of spleen tissue can be exposed to SHG Nd:YAG laser (magnification x 100).

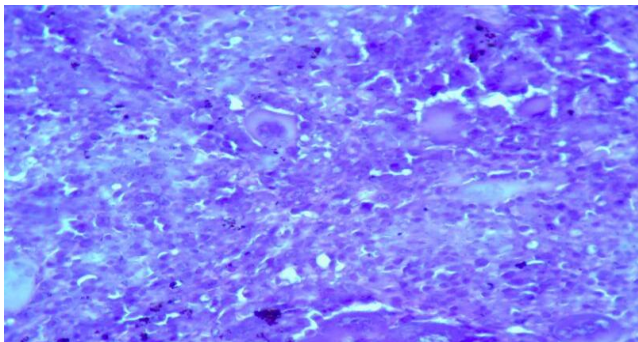


Figure (23): section of spleen tissue can be exposed to SHG Nd:YAG laser (magnification x 200).

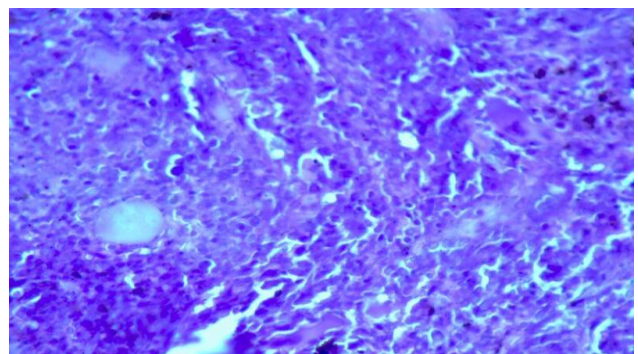


Figure (24): section of spleen tissue can be exposed to SHG Nd:YAG laser (magnification x 400).

From these figures, the sections of spleen are showing certain necrosis of parenchymal tissue. Also, the section in figure (24) is showing the presence of megakaryocyte cells infiltrate.

c) 785 nm LASER DIODE:

When spleen tissue is exposed to N-IR laser diode of 785 nm wavelength at a distance of 1 cm, and with time of (5 sec), then the tissue of the spleen is showing in figure (25) and figure (26) and figure (27).

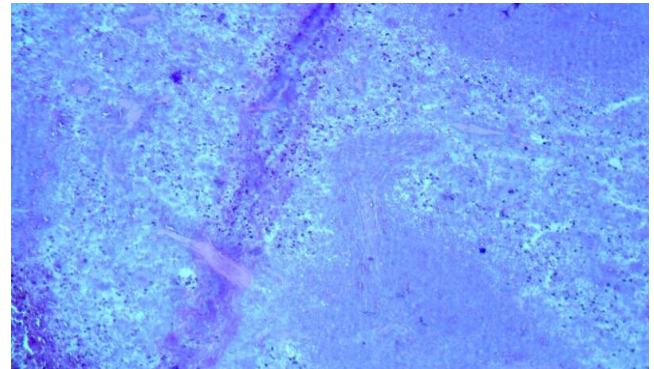


Figure (25): section of spleen tissue can be exposed to 785 laser diode (magnification x 100).

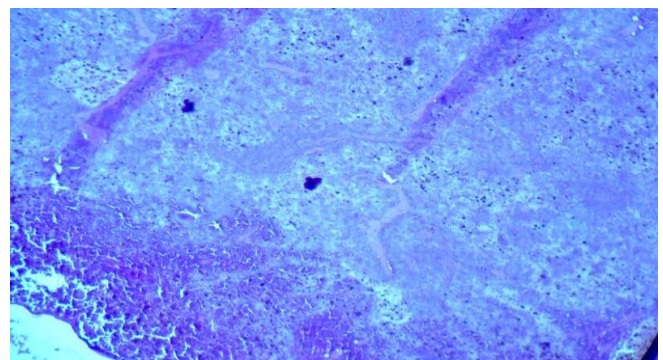


Figure (26): section of spleen tissue can be exposed to 785 laser diode (magnification x 200).

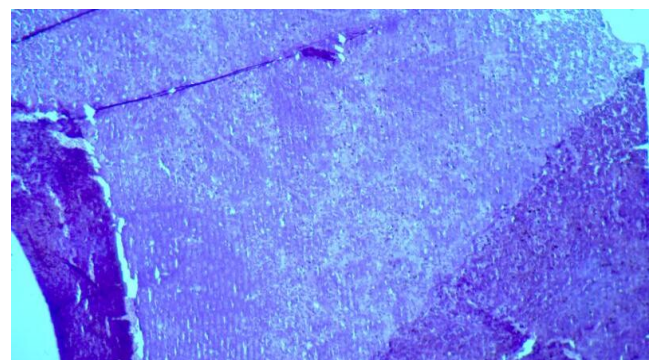


Figure (27): section of spleen tissue can be exposed to 785 laser diode (magnification x 400).

From these figures, the sections are showing the degeneration and necrosis of splenic parenchymal tissue.

d) CO₂ LASER:

The spleen's tissue is shine with a CO₂ laser at a distance of (25 cm) and with time of (5 sec), which the exposed tissue is shown in figure (28) and figure (29) and figure (30).

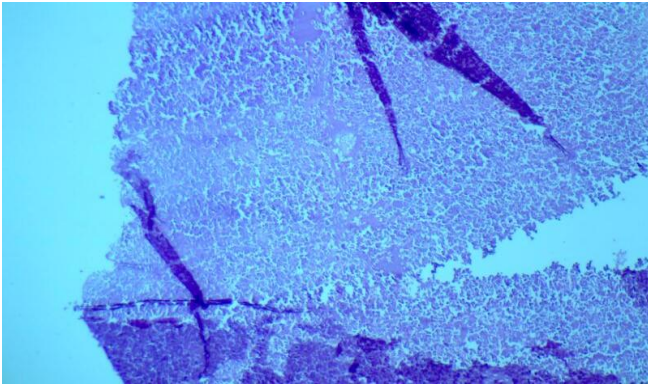


Figure (28): section of spleen tissue can be exposed to CO₂ laser (magnification x 400).

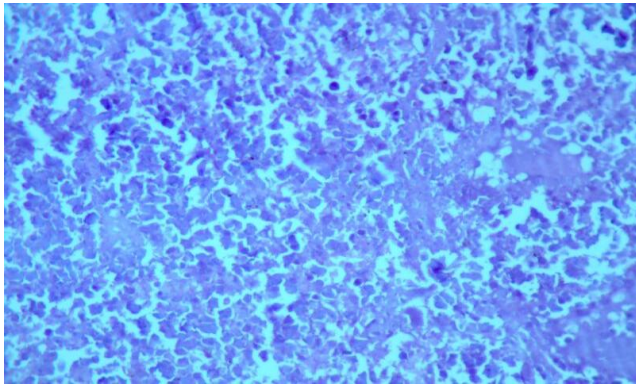


Figure (29): section of spleen tissue can be exposed to CO₂ laser (magnification x 400).

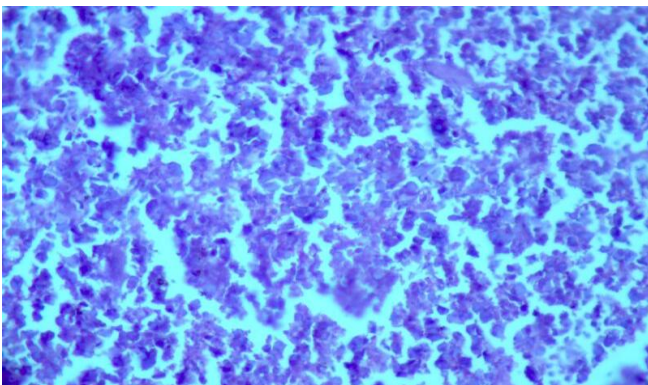


Figure (30): section of spleen tissue can be exposed to CO₂ laser (magnification x 400).

V. DISCUSSION:

The absorption of light depends on concentration and absorption spectra of specific molecules in the tissue. The difference in the spectral absorption properties of different molecules permits selective damage to specific components of a target tissue. In this study and from the work in the laboratory, we can observe and this was clearly that the surface of the tissue was affected with the laser differently from one laser to another and from tissue to another. When The 405 nm laser diode, SHG Nd:AG laser or 785 nm laser were exposed to the tissue, the surface of the tissue was effected by these lasers, as these lasers caused marks with different sizes from laser to other. While in case of CO₂ laser, the result was different from the other lasers as it was causing a black fringe. In the Histological study, it can be found that when the biological tissue was exposed to different laser

wavelength and different power or energy, this tissue can be affected differently by these lasers. Also, the same laser can effect differently from one tissue to another.

In the case of liver, it can be found that the SHG Nd:YAG can be the most affective. While in case of spleen, it can be found that the different types of lasers will cause degeneration and necrosis of parenchymal splenic tissue, but with different levels.

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